APPENDIX A:
STUDY PLAN FOR DETERMINING INTERLABORATORY VARIABILITY OF THE EPA SHORT-TERM CHRONIC AND ACUTE WHOLE EFFLUENT TOXICITY TEST METHODS

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SECTION 1: INTRODUCTION AND BACKGROUND

The Clean Water Act (CWA) requires the U.S. Environmental Protection Agency (EPA) to promulgate guidelines establishing test procedures for data gathering and compliance monitoring under National Pollution Discharge Elimination System (NPDES) permits. Within EPA, the Office of Water (OW) publishes test procedures for the analysis of wastewater, freshwater, and marine waters subject to NPDES compliance monitoring requirements. These test procedures are specified at title 40, part 136 of the *Code of Federal Regulations* (CFR). On October 16, 1995, EPA promulgated a final rule approving the use of whole effluent toxicity (WET) test methods to protect aquatic life in NPDES compliance monitoring (60 FR 53529). Whole effluent toxicity is defined as the aggregate toxic effect of an effluent or receiving water measured directly with a toxicity test. The Agency-approved WET test methods are listed at 40 CFR §136.3, Table IA. These WET test procedures employ a suite of standardized freshwater, marine and estuarine plants, invertebrates, and vertebrates to measure acute and short-term chronic toxicity. The EPA-approved WET methods resulted from many years of development and testing by EPA, states, municipalities, academia, and the regulated community. As part of a settlement agreement to resolve a judicial challenge to the WET methods rule, EPA will conduct interlaboratory variability studies of twelve of the seventeen promulgated WET methods.

The interlaboratory variability study of the five acute and seven short-term chronic WET methods will be performed in three rounds. EPA shall design the interlaboratory variability studies to, among other things, quantify interlaboratory variability, i.e., to determine an estimate of precision, including, at a minimum, a coefficient of variation, for each test endpoint, as well as to determine the rate at which participating laboratories successfully completed tests initiated and the rate at which the tests indicate toxicity is present when measuring reagent water, also known as “blanks.” Freshwater tests will be conducted in Round 1 and marine tests will be conducted in Rounds 2 and 3. The WET methods and the round in which they will be performed in the interlaboratory study are listed in Table 1.

Table 2 identifies the test duration and test endpoints for the five acute and seven short-term chronic methods included in the interlaboratory study.

The general design of the interlaboratory variability study is as follows:

- A total of 12 WET methods (five freshwater tests and seven marine tests) will be conducted (See Tables 1 and 2 below).

- A minimum of nine “participant” laboratories will be selected to perform each WET method.

- At least one “referee” laboratory for Round 1 and at least one referee laboratory for both Round 2 and 3 will be required to collect and prepare blind test samples and distribute them to the participant laboratories, and conduct the tests.

- Each participant laboratory will perform four tests per method as part of the interlaboratory variability study process, therefore a total of 36 “test values” will be determined for each method at a minimum.

- The four WET tests will be conducted with four blind test samples. For the purpose of this study plan, a “test sample” is a single bulk sample preparation (i.e, matrix, recipe) that is provided to a participant laboratory. Aliquots of the single bulk sample will be used for test initiation and renewal(s) for the WET test method under study.

Table 1 - WET Methods Included in the Interlaboratory Variability Study

Round 1 - Freshwater Tests

(1) Fathead Minnow, *Pimephales promelas*, Acute Test[1]
(2) Method 1000.0: Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test[2]
(3) Cladoceran, *Ceriodaphnia dubia*, Acute Test[1]
(4) Method 1002.0: Cladoceran, *Ceriodaphnia dubia*, Survival and Reproduction Test[2]
(5) Method 1003.0: Green Alga, *Selenastrum capricornutum*, Growth Test[2]

Round 2 - Marine Tests

(1) Sheepshead minnow, *Cyprinodon variegatus*, Acute Test[1]
(2) Method 1004.0: Sheepshead minnow, *Cyprinodon variegatus*, Larval Survival and Growth Test[3]
(3) Inland silverside, *Menidia beryllina*, Acute Test[1]
(4) Method 1006.0: Inland silverside, *Menidia beryllina*, Larval Survival and Growth Test[3]
(5) Mysid, *Holmesimysis costata*, Acute Test[1]

Round 3 - Marine Tests

(1) Method 1007.0: Mysid, *Mysidopsis bahia*, Survival, Growth, and Fecundity Test[3]
(2) Method 1009.0: Red Macroalga, *Champia parvula*, Reproduction Test[3]

[1]USEPA, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition, EPA-600-4-90-027F, August 1993
[2]USEPA, *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms*, Third Edition, EPA-600-4-91-002, July 1994
[3]USEPA, *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms*, Second Edition, EPA-600-4-91-003, July 1994
NOTE: EPA shall conduct interlaboratory variability studies using the specific test protocols promulgated at 40 CFR Part 136, including, as appropriate, reference to EPA guidance entitled "Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods" dated April 10, 1996 from Tudor T. Davies, EPA Office of Science and Technology to EPA Water Management Division Directors and EPA environmental Services Division Directors.

- Four freshwater or four marine test samples (depending on whether a freshwater or marine test is being conducted) will be included. Both real-world (field collected) and synthetic (laboratory prepared) freshwater and marine test samples will be prepared.

- Replicate (i.e., duplicate) test samples will be included in the four blind test samples distributed to each participant laboratory for each method.

- Additional WET tests will be conducted in the referee laboratories for each method in the study.

- Each participating laboratory shall be required to report all data obtained during the course of testing, including the response of control samples.

- EPA will assess intralaboratory and interlaboratory variability of the 12 WET methods through the determination of the coefficient of variation (CV) for the LC_{50} and IC_{25} endpoints and the range of values for the NOEC endpoints for each method in the study. The study is also designed to provide data on the rate at which participating laboratories successfully completed tests initiated and to the rate at which the tests indicate "toxicity" is present when measuring non-toxic samples.

Table 2 - Twelve Acute and Short-Term Chronic WET Methods

Round	EPA Methods for the Interlaboratory Variability Study	Acute Tests		Short-Term Chronic Tests			
		Survival LC_{50}	Test Duration (Hours)	Survival LC_{50} NOEC	Growth IC_{25} NOEC	Reprod IC_{25} NOEC	Test Duration (Days)
1	Fathead Minnow, *Pimephales promelas* - Acute Test	X	96				
1	Method 1000.0: Fathead Minnow, *Pimephales promelas*, Larval Survival & Growth Test			X	X		7
1	Cladoceran, *Ceriodaphnia dubia* - Acute Test	X	48				
1	Method 1002.0: Cladoceran, *Ceriodaphnia dubia*, Survival & Reproduction Test			X		X	8[1]
1	Method 1003.0: Green Alga, *Selenastrum capricornutum*, Growth Test				X		4
2	Sheepshead Minnow, *Cyprinodon variegatus*, Acute Test	X	96				
2	Method 1004.0: Sheepshead Minnow, *Cyprinodon variegatus* - Larval Survival & Growth Test			X	X		7
2	Inland Silverside, *Menidia beryillina* - Acute Test	X	96				
2	Method 1006.0: Inland Silverside, *Menidia beryillina*, Larval Survival and Growth Test			X	X		7
2	Mysid, *Holmesimysis costata* - Acute Test[2]	X	96				
3	Method 1007.0: Mysid, *Mysidopsis bahia* - Survival, Growth, and Fecundity Test			X	X	X	7
3	Method 1009.0: Red Macroalga, *Champia parvula*, Reproduction (cystocarp production) Test					X	7 - 9[3]

[1] The *C. dubia* test acceptability criteria states that the test is complete when 60% of controls have 3 broods (approximately 7 days); for purposes of this study, all tests will continue for 8 days and each laboratory must carefully distinguish and carefully record the number of broods (see Section 4.5.4 in this study plan).

[2] The EPA-approved acute test with *Holmesimysis costata* will be performed using the acute test procedures for *Mysidopsis bahia* and test conditions optimized for *H. costata*.

[3] *C. parvula* are exposed to test substance for two days, followed by a 5-7 day recovery period in control water.

The remainder of this study plan describes the design of EPA's interlaboratory variability study of 12 WET test methods. In the performance of each WET method, participating laboratories shall follow the specific instructions that EPA (or EPA's authorized representative) shall provide to perform the testing in accordance with their routine laboratory practices using the applicable test methods from the WET final rule. Additionally, EPA will provide all laboratories interested in the referee or participant laboratory role with detailed statements of work (SOWs) that articulate the specific tasks, instructions, deliverables, and turnaround requirements associated with each role. Laboratories selected as referee laboratories for a specific testing round, may not subsequently fill the participant laboratory role for that same testing round. EPA may modify this study plan, the SOWs, or any specific instructions prior to or during the performance of the interlaboratory study.

SECTION 2: OBJECTIVES

The primary objectives of this study are to (1) generate data to characterize the intralaboratory and interlaboratory variability (i.e., precision) of the 12 WET methods for each method in the study, (2) obtain data on the rate at which participating laboratories successfully completed WET tests initiated, and (3) generate data on the rate at which WET tests indicate "toxicity" is present when measuring non-toxic samples.

This study will be conducted in five phases designed to accomplish the overall study objectives. These phases, and the basic objectives associated with each phase, are shown in Table 3.

Table 3 - Five Phases of the Variability Study

Phase	Objectives
1	• Identify potential referee and participant laboratories to support the study
2	• Prequalify and select referee laboratories for Phases 3, 4, and 5 • Prequalify and select participant laboratories for Phase 5 of the study
3	• Identify real-world and synthetic test samples that meet the needs of the study and can be prepared and distributed efficiently and cost-effectively for use in Phase 5
4	• Prepare real-world and synthetic test samples for use by referee and participant laboratories in Phase 5 • Minimize variability between samples prepared for and distributed to each of the Phase 5 laboratories • Distribute blind test samples to all qualified laboratories for initial use within 36 hours of individual sample shipment from the referee laboratories
5	• Obtain at least six sets of usable data from the participant laboratories for each WET method using real-world and synthetic test samples to evaluate precision of the test methods, the rate at which laboratories successfully completed tests initiated, and the rate at which the tests indicate "toxicity" is present when measuring non-toxic samples • Obtain data in the referee laboratories for each method using real-world and synthetic test samples to obtain additional intralaboratory test data and comparability data with test samples analyzed in the participant laboratories.

Four data quality objectives (DQOs) have been identified as necessary to ensure that data produced will meet the study objectives described above. These are:

(1) All data produced in the study must be generated in accordance with the analytical and quality assurance/quality control (QA/QC) procedures defined in this study plan and the following documents:

- *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms*, Second Edition, EPA-600-4-91-003, July 1994; (hereinafter referred to as the "Marine Chronic Methods Manual").
- *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms*, Third Edition, EPA-600-4-91-002, July 1994, (hereinafter referred to as the "Freshwater Chronic Methods Manual").
- *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, Fourth Edition, EPA-600-4-90-027F, August 1993, (hereinafter referred to as the "Acute Methods Manual"); including errata sheet.

- Memorandum from Tudor Davies, Office of Science and Technology, USEPA dated April 10, 1996, Subject of the memorandum is: Clarifications Regarding Flexibility in 40 CFR Part 136 Whole Effluent Toxicity (WET) Test Methods.

The first three documents are referred to collectively as the "methods manuals" throughout this document. The test requirements in Sections 4.5.3 and 4.5.4 of this study plan and the specific instructions provided by EPA will define the allowable flexibility in the WET methods included in this study. This study plan and the specific instructions will address items agreed to by EPA in the settlement that are currently not specified in the methods manuals.

(2) All test results from controls must meet the required test acceptability criteria (i.e. survival, minimum growth, minimum offspring/reproduction, average dry weight) specified in the methods manuals and Section 4.5.4 of this study plan to be considered valid. All *Ceriodaphnia dubia* tests will be conducted for 8 days and broods will be counted for the reproductive days of the test (see Section 4.5.4).

(3) Test parameters must meet the range of chemical and physical test conditions (such as temperature, hardness, alkalinity, ammonia, conductivity, pH, salinity, etc.) outlined in the appropriate methods manual and as detailed in Section 4.5.3 of this study plan.

(4) All calculations and data produced in this study must be capable of being verified through an independent review of the final data package by an analyst familiar with WET testing.

To meet these DQOs, each participating laboratory will be required to have a comprehensive QA program in place and operating throughout this study.

SECTION 3: STUDY MANAGEMENT

The study described in this document will be jointly funded by the EPA Office of Water Engineering and Analysis Division (EAD) and Permits Division (PD). The study will be managed by EAD's Analytical Methods Staff (AMS) with assistance from EPA's Office of Research and Development (ORD). Day-to-day management and coordination of study activities will be performed by the contractor-operated Sample Control Center (SCC) under AMS guidance.

Laboratories participating in the interlaboratory study will include EPA, state, academic, municipal, industrial and/or private laboratories. Laboratories will not be required to participate in the interlaboratory variability of all twelve methods under study. To the extent possible, laboratory participation will be voluntary. In addition to voluntary laboratory support, EPA will procure additional laboratory services for this study through government laboratory contracting vehicles.

Drop-out rates for laboratories and late data delivery dates are likely to occur in interlaboratory studies, especially studies that incorporate voluntary labs. Therefore, a minimum of nine participant laboratories are desired to produce a minimum of six data sets for each method under study in Phase 5. Initiation of Phase 5 will be delayed until at least nine participant laboratories have been prequalified for each method. Multiple referee laboratories will likely be selected to participate in this study. Initiation of Phases 3 and 4 will be delayed until a sufficient number of referee laboratories have been selected to support the interlaboratory study. The total number of participant and referee laboratories participating in this study, will be dependent upon laboratory capability, laboratory availability, cost, and scheduling constraints.

SCC will coordinate all activities related to field sampling, preparation and shipment of blind test samples, and laboratory analysis, and will review, validate, and statistically analyze all data. SCC will draw conclusions from the results and produce a final report for EPA review.

SECTION 4: TECHNICAL APPROACH

At least nine participant laboratories will conduct each test method to be evaluated in the interlaboratory variability study. At least one referee laboratory for Round 1 and at least one referee laboratory for both Round 2 and 3 will be required to collect and prepare blind test samples and distribute them to the participant laboratories, and conduct the tests. EPA shall assure that all of the laboratories selected for participation in the interlaboratory study are representative of laboratories throughout the United States that routinely conduct WET testing for permittees and shall attempt to maximize the number of qualifying laboratories participating in the interlaboratory study.

EPA shall identify laboratories qualified for participation in the interlaboratory study. Laboratories participating in the study must demonstrate satisfactory quality assurance and quality control (QA/QC) based on QA/QC procedures in the test manuals. The minimum QA/QC prequalification requirements shall include the acceptable control charts (i.e. cusum charts) using reference toxicants, meeting of test conditions and test acceptability criteria, and the application of appropriate statistical analyses for each test and test endpoint for which the laboratory would perform in the interlaboratory study. These laboratory qualification criteria are incorporated into this interlaboratory variability study design (see Section 4.2 and 6.1 - 6.3 below). Additional participant laboratories which meet those qualifications shall be allowed to take part in the interlaboratory study provided that the costs of the analysis associated with such participation shall be the sole obligation of the additional participant laboratory.

4.1 Phase 1 - Identification of Referee and Participant Laboratories

The purpose of Phase 1 is to identify potentially qualified referee and participant laboratories for this study. Laboratories solicited to participate will be identified from a variety of sources, including EPA and state environmental agencies, the Society of Environmental Toxicology and Chemistry (SETAC), reviews of the public literature, *the Directory of Environmental Laboratories*[1], and EPA's Discharge Monitoring Report Quality Assurance (DMRQA) list of laboratories conducting testing for the DMRQA program.

4.2 Phase 2 - Selection of Referee and Participant Laboratories for Phase 5

The purpose of Phase 2 is to determine the capability of potential referee and participant laboratories to take part in the study through an evaluation of laboratory experience and proficiency in WET testing. Laboratory prequalification requirements, rejection criteria, and turnaround requirements are described further in Section 6 of this study plan.

Laboratories may choose to prequalify to preform (and/or support) one or more of the twelve WET methods. The entire prequalification process must be completed for each WET method potential laboratories are interested in performing. As noted previously, laboratories selected as referee laboratories for a specific testing round, may not fill the participant laboratory role for that same testing round. EPA will forward a bid solicitation package to potential laboratories identified in Phase 1 that includes the following documents: (1) laboratory prequalification document, (2) SOW, including a preliminary study schedule, and (3) laboratory bid sheet. Phase 2 prequalification will be divided into two parts. Part I

[1] *Directory of Environmental Laboratories*, DynCorp, 1996.

consists of historical WET testing experience and proficiency as described in Section 6.2. Part II consists of the prequalification test and requests general and WET method specific information regarding the capabilities of potential laboratories (see Section 6.3).

To prepare a complete package for Part I, all laboratories must address the prequalification requirements and attach all required documentation (including historical QA/QC control charts), provide an explanation for the omission of any requisite information, complete the laboratory bid sheet, and forward the material to EPA for consideration within 15 days of receipt of the solicitation package. Laboratories that meet the requirements of Part I (Section 6.2), or provide a satisfactory explanation for not fulfilling specific Part I requirements, will be given sufficient notice that they have been qualified to participate in the Part II prequalification (Section 6.3).

For Part II, participant laboratories meeting the requirements of Part I will be forwarded a single prequalification reference toxicant sample for each method they intend to perform in the interlaboratory study. The reference toxicant samples will provide sufficient volume for the required physical and chemical tests, dilution concentrations, and replicates. Detailed instructions for performing the prequalification tests will be provided to potential participant laboratories along with the prequalification sample. Laboratories will be required to initiate analysis within 24 hours of prequalification sample receipt. After analyzing the prequalification samples, laboratories must report all raw and summarized data to EPA within 30 days of sample receipt. All prequalification tests will be performed at the laboratory's cost. In lieu of analyzing a prequalification sample, referee laboratories approved to participate in Part II will be required to submit at least three client recommendations per WET method they are seeking to support in the study. These recommendations must come from clients for whom they have conducted these same WET tests in the past.

All prequalification results submitted by participant laboratories with each WET method will be evaluated for outliers as a group. Laboratories will be given the opportunity to explain outlier values. Each laboratory that successfully completes each test method, meets the test acceptability criteria, and provides an adequate explanation of any outlier data with the prequalification sample will be considered acceptable to participate in the study. Participant laboratories with acceptable results will be selected based on bid cost. EPA will support the cost of testing for nine participant laboratories for each method in Phase 5. EPA will evaluate data generated by additional prequalified participant laboratories who agree to take part in the study at their own cost. EPA will select at least two referee laboratories to support Phases 3, 4, and 5 of the study based on a review of their overall qualifications as demonstrated in Part I and II of prequalification in toto.

4.3 Phase 3 - Selection of Test Samples

In Phase 3, EPA will select test samples that exhibit a range of toxicity for use in Phase 5. As mentioned in Section 1, a “test sample” is a single bulk sample preparation (i.e, matrix, recipe) that is provided to a participant laboratory. Aliquots of the single bulk sample will be used for test initiation and renewal(s) for the WET test method under study. The agency will select test samples that reflect the precision of the tests and not the variability of the toxicant. Consideration of common toxicants in most effluent discharges will be made and toxicants may be different for freshwater and marine testing.

EPA shall randomly distribute “blind” test samples to each laboratory for evaluation. The test samples distributed shall include some combination of: reference toxicants (of known chemical composition); industrial and/or municipal wastewater effluent (of unquantified chemical composition); ambient receiving water; and method “blanks,” i.e., moderately hard reagent water as explained in the test method manuals.

The combinations of blind test samples may include more than one test sample of any given sample type. Neither EPA, EPA's authorized representatives, nor selected referee laboratories shall disclose the nature, number, or composition of any of the various samples distributed to laboratories participating in the studies. A total of four freshwater and four marine test samples that represent a range of toxicity will be included in the study. Both "real-world" (field collected) and synthetic (laboratory prepared) test samples for freshwater and marine tests will be prepared for use in the interlaboratory study. The specific instructions provided to referee laboratories and the referee laboratory SOW will provide detailed directions about the types of test samples that should be prepared for the study.

As described in Table 1, the Phase 5 interlaboratory study is divided into three separate testing rounds; freshwater tests will be conducted in Round 1 and marine tests will be conducted in Rounds 2 and 3. A total of four test samples will be prepared and distributed by the referee laboratories as blind samples to each participant laboratory for each method they are performing in Phase 5. Duplicates of selected test samples will be included in the four blind samples distributed to the participant laboratories for each method. Additionally, single and duplicate sample aliquots of selected test samples will be prepared and retained in the referee laboratory for analysis.

4.4 Phase 4 - Collection, Preparation, and Distribution of Test Samples

The referee laboratories will obtain all equipment, supplies, and or materials needed to meet the requirements of Phases 3 and 4.

4.4.1 Collection of Real-World Samples

The referee laboratories will collect real-world samples for preliminary testing during sample selection and the Phase 5 interlaboratory study. There will be five separate "sampling periods" as described in Table 4. Samples shall be collected in accordance with the procedures provided in specific instructions provided to the referee laboratories, the referee laboratory SOW, and Section 8 of the methods manuals. All real-world samples will be collected as grab samples. The same type of sampling equipment and procedures will be used to collect individual matrices to minimize sample variability. Ideally, grab samples be collected using a peristaltic pump. The pump tubing should be thoroughly rinsed with source water prior to sampling. Real-world water volume will be added to a sufficient number of "intermediate" containers to provide a total "bulk" volume that meets the needs of each collection period.

The referee laboratory shall assign an episode number to track each sampling event. All samples shall be identified with a five-digit EPA sample number and documented on EPA traffic reports. Sample numbers, sample labels, and EPA traffic reports will be provided to the referee laboratory by SCC along with detailed instructions for sample documentation.

The referee laboratory SOW and specific instructions provided to the referee laboratories will give detailed instructions about the volume of each real-world test samples that should be collected for the WET methods included in this study.

Table 4 - Collection of Real-World Samples

Sample Period	Testing Round	Type of Test Sample Collected	Approximate Sampling Period[1]	Use of Samples
1	Preliminary Testing	Freshwater and Marine	November 1998-January 1999	Phase 3 - Sample aliquots for preliminary testing
2		Freshwater and Marine	November 1998 - January 1999	Phase 3 - Sample aliquots for preliminary testing
3	1	Freshwater	March - April 1999	Phase 5 - Test samples for Round 1
4	2	Marine	May - June 1999	Phase 5 - Test samples for Round 2
5	3	Marine	July - August 1999	Phase 5 - Test samples for Round 3

[1] Approximate sampling period based on October 1998 preliminary schedule. A final study schedule will accompany referee laboratory bid acceptance notification.

4.4.2 Preparation of Test Samples

The referee laboratories will prepare test samples for use in prequalification, preliminary testing, and the Phase 5 interlaboratory study. Ampules of a selected reference toxicant will be prepared for use in prequalification testing. Sufficient volumes of the test samples will be prepared and retained in the referee laboratory for preliminary analyses during samples selection and WET testing in Phase 5. For Phase 5, the referee laboratory will prepare four different freshwater samples for Round 1 and four marine test samples for Rounds 2 and 3. Samples for all tests will be prepared in containers appropriate for the individual test samples (e.g., ampules, glass containers, CUBITANERs). Separate tanks that have been cleaned and rinsed should be used for the bulk samples of each real-world and synthetic test sample prepared in the referee laboratories. All tanks will then be thoroughly mixed on a daily basis to homogenize the contents. Sufficient bulk volume shall be prepared during each sample preparation period to meet the sample renewal needs for the duration of each method under study.

Prior to preparing individual samples for in-house testing or distribution to participant laboratories, all containers will be rinsed with the appropriate test sample. To minimize the loss of sample components due to volatilization, aliquots of the bulk samples will be poured from the tanks into appropriate sample containers until they are completely filled, leaving no air space between the contents and the lid. The date and time of sample preparation shall be indicated clearly on the sample label. After preparation, individual samples will be maintained at 4°C ± 2°C prior to shipment. The referee laboratory shall identify each test sample with a five-digit EPA sample number and properly document it on an EPA traffic report. SCC will provide the referee laboratories with samples numbers, samples labels, and EPA traffic report forms.

The number of individual samples (aliquots) for test initiation and renewal (if required) that shall be prepared for the performance of each WET method in nine participants laboratories is provided in Table 5. More samples will be needed for additional volunteer laboratories. Table 6 describes the test sample type and the corresponding approximate sample preparation and distribution period.

All samples will be shipped to the performing laboratories as described in the methods manuals and Section 4.4.3 of the this study plan. A description of the specific test sample volumes prepared for each method in each testing round will be outlined in the referee laboratory SOW and specific instructions provided to the referee laboratories.

Table 5 - Total Number of Sample Aliquots Prepared for Study

Sample Prepared For:	Number of Test Samples Per Method[1]	Number of Aliquots Required per Test	Number of Labs	Total Number of Aliquots Prepared[2]
Prequalification Samples:	N/A	N/A	N/A	240
Samples for Interlaboratory Study:				
Round 1 - Freshwater Tests				
Fathead Minnow, *Pimephales promelas*, 96-hour Acute Test	4	2	9	72
Cladoceran, *Ceriodaphnia dubia*, 48-hour Acute Test	4	1	9	36
Method 1000.0: Fathead Minnow, *Pimephales promelas*, 7-day Larval Survival and Growth Test	4	3	9	108
Method 1002.0: Cladoceran, *Ceriodaphnia dubia*, 7-9-day Survival and Reproduction Test	4	3	9	108
Method 1003.0: Green Alga, *Selenastrum capricornutum*, 4-day Growth Test (with and without EDTA)	4	1	9	36
Round 2 - Marine Tests				
Sheepshead minnow, *Cyprinodon variegatus*, 96-hour Acute Test	4	2	9	72
Inland silverside, *Menidia beryllina*, 96-hour Acute Test	4	2	9	72
Method 1004.0: Sheepshead minnow, *Cyprinodon variegatus*, 7-day Larval Survival and Growth	4	3	9	108
Method 1006.0: Inland silverside, *Menidia beryllina*, 7-day Larval Survival and Growth Test	4	3	9	108
Mysid, *Holmesimysis costata*, 96-hour Acute Test	4	2	9	72
Round 3 - Marine Tests				
Method 1007.0: Mysid, *Mysidopsis bahia*, Survival, 7-9 day Growth, and Fecundity Test	4	3	9	108
Method 1009.0: Red Macroalga, *Champia parvula*, 3-day Reproduction Test	4	1	9	36
Total Number of Samples Prepared for the Study				**936**

[1] Four test samples will be distributed to each participant laboratory for each WET method.

[2] This is the minimum number of aliquots to be tested; does not include volunteer laboratories

Table 6 - Test Sample Preparation and Distribution

Testing Round	Test Sample Type	Approximate Sample Preparation and/or Distribution Period[1]	Test Sample Number[2]
Preliminary Sample Preparation	Freshwater and Marine	November 1998 - January 1999	Samples for preliminary tests
		November 1998 - January 1999	Samples for preliminary tests
		January - February 1999	Prequalification tests
1	Freshwater	March - April 1999	Sample 1
			Sample 2
			Sample 3
			Sample 4
2	Marine	May - June 1999	Sample 1
			Sample 2
			Sample 3
			Sample 4
3	Marine	July - August 1999	Sample 1
			Sample 2
			Sample 3
			Sample 4

[1] Approximate sample preparation period based on October1998 preliminary schedule. A final study schedule will accompany the referee laboratory bid acceptance notification.

[2] Test sample refers to multiple sample aliquots for test initiation and renewal over the duration of the exposure period for methods requiring daily sample renewal.

4.4.3 Distribution of Test Samples

The referee laboratories will ship test samples as blind samples to the laboratories participating in the interlaboratory study according to the approximate time frame described in Table 6 above. All samples will be distributed to participant laboratories in accordance with the sample shipment procedures specified in Section 8 of the methods manuals. The referee laboratories will ship all blind samples for an individual method to the participant laboratories in exactly the same quantity and type of container. SCC will provide the referee laboratories with a participant laboratory list and overnight carriers shipping forms and billing number for shipment of samples to the laboratories participating in the study. The list of participant laboratories will include addresses and contacts, as well as specifications for the types of samples each participant laboratory is to receive.

The referee laboratory will complete the traffic reports, using the label information discussed above, pack samples in coolers as described in the methods manuals, complete the airbills, and ship samples to the participant laboratories using priority overnight service. The referee laboratory shall immediately notify SCC of sample shipment, including airbill numbers. Following completion of the study, participant laboratories will be required to return coolers to the referee laboratories.

The detailed description of the sample distribution procedures will be provided in the referee laboratory SOW and specific instructions provided to the referee laboratories.

4.5 Phase 5 - Interlaboratory Study

The general conduct of the interlaboratory study will proceed as described in Section 1 of this study plan. Round 1 will include the acute and short-term chronic *Ceriodaphnia dubia* and *Pimephales promelas* tests and the short-term chronic *Selenastrum capricornutum* test. Round 2 includes the acute and short-term chronic *Cyprinodon variegatus* and *Menidia beryllina* tests and the acute *Holmesimysis costata* test. Round 3 includes the short-term chronic *Mysidopsis bahia* and *Champia parvula* tests.

4.5.1 Study Initiation

Following prequalification, EPA will notify referee and participant laboratories that have been selected to take part in the variability study. This notification will be accompanied or followed by a final study schedule. EPA will provide adequate time for laboratories to prepare for study initiation. The referee laboratories will provide traffic report forms with each blind test sample sent to the participant laboratories to document sample shipment and receipt. Upon receipt of the samples, laboratories will be responsible for determining that the samples arrived in satisfactory condition and for documenting receipt of the samples and any problems noted on the EPA traffic report forms. Laboratories will be required to retain a copy of the completed traffic report form and return a copy to SCC. If individual test samples are unusable or not received, the participant laboratories must contact the appropriate referee laboratory and/or SCC for problem resolution. Referee laboratories must clearly document all test samples retained for WET testing.

4.5.2 Preliminary Study Schedule

The interlaboratory study will be conducted from approximately March to August 1999. Table 7 describes the preliminary sample receipt and test initiation schedule for the interlaboratory study. *Note: This is a preliminary schedule for planning purposes only; a final study schedule will be provided to participant laboratories with bid acceptance notification*.

4.5.3 General Testing Requirements

Each participant laboratory will receive four blind test samples for each method they are approved to perform. Additionally, single and duplicate sample aliquots of real-world and synthetic test samples will be analyzed in the referee laboratories. All samples should be treated as if they are effluent samples being tested for compliance monitoring purposes. Except where indicated in Sections 4.5.3 and 4.5.4 of this study plan, the SOWs, and specific instructions provided to laboratories, each test will be conducted in accordance with the general guidance and method specific requirements for effluent testing included in the methods manuals.

EPA acknowledges that the promulgated WET methods distinguish between requirements (required are the compulsory terms “must” and “shall”) and recommendations and guidance (use of discretionary terms “should” and “may”). These terms indicate that the analyst has flexibility to optimize successful test completion and when standardization is necessary to assure the predictability of the methods to provide reliable results. Additionally, the method manuals allow variations of the methods which are typically fixed in the permit; therefore for the purposes of this study, a set of variables will be defined by EPA (for example, dilution water, salinity, and acute test duration).

Additional WET test requirements and general requirements listed in the methods manuals of special note are provided below:

(1) Personnel that conduct the prequalification, preliminary, and Phase 5 tests must be the same personnel that routinely conduct the WET tests at that laboratory facility. Personnel conducting the tests must be identified clearly and consistently in records.

Table 7 - Participant Laboratory Sample Receipt and Test Initiation Schedule

Testing Round	Test Sample Number[1]	Approximate Sample Receipt and Testing Period
1	1	March 1999
	2	April 1999
	3	
	4	
2	1	May 1999
	2	
	3	June 1999
	4	
3	1	July 1999
	2	
	3	August 1999
	4	

[1] Test sample refers to multiple sample aliquots for test initiation and renewal over the duration of the exposure period for methods requiring sample renewal.

(2) The time between shipment of an individual blind test sample by the referee laboratory and initial use of the sample by participant laboratories must not exceed 36 hours. The date and time of sample preparation will be identified on each blind test sample. Deviations of any nature must be discussed with the referee laboratory and/or SCC immediately.

(3) Physical and chemical properties of monitoring of the test samples must be in the ranges specified in this study plan, specific instructions, and the methods manuals. Method specific instructions for any adjustments to the test samples prior to sample use will be provided to the testing laboratories following guidance from the methods manuals.

(4) Measurement of test conditions (e.g., pH, conductivity or salinity, total alkalinity, total hardness, and dissolved oxygen) shall be performed for each method by the participant laboratories using the guidance in the methods manuals. Additionally, analyses of total residual chlorine and ammonia may be required in the specific instructions.

(5) Appropriate dilution and control water (synthetic freshwater or salinity of marine water) will be specified for each test method. The identity and preparation procedures for appropriate waters will be provided in specific instructions given to laboratories and Section 7 of the methods manuals.

(6) All WET tests are to be definitive tests with a minimum of five test concentrations and dilution factor of 0.5 or greater and control as described in the specific instructions.

(7) All tests must be conducted using the number of replicates and number of test containers per concentration as specified in Section 4.5.4 below.

(8) Test chambers must be identical within a test and made of material allowed by the specific method.

(9) Test vessels shall be randomized in accordance with the method manuals. In addition, block randomization and use of known parentage will be required for the *Ceriodaphnia dubia* survival and reproduction test as described in Method 1002. This guidance will be reiterated in the specific instructions provided to the selected referee and participant laboratories. The Agency plans to amend Method 1002 (*Ceriodaphnia dubia* Survival and Reproduction test) to require that test organisms be allocated among test replicates so that offspring of each female are evenly distributed among test replicates ("blocking-by-parentage"). In addition, while Method 1002.0, which would otherwise be terminated after 3 broods according to Section 13.12.1 of that Method, each *Ceriodaphnia dubia* shall be conducted for 8 days (through to completion), with endpoints (including number of young per day and number of broods at each recording interval) noted at the end of the sixth, seventh and eighth day (specifically, at 144 hours, at 168 hours, and at 192 hours, respectively, from test initiation), in order to assess the effect of that test acceptance criterion on test results. No test shall be terminated prior to the eighth day for any reason, including a failure to meet test acceptance criteria. Finally, in the conduct of Method 1002.0, test organisms shall be allocated among test replicates so that the offspring of each female are evenly distributed among test replicates ("blocking-by-parentage").

(10) Daily observation of mortality and removal of dead organisms for each test is required, except for the *Selenastrum* and *Champia* tests. Daily young counts are required for the *Ceriodaphnia dubia* survival and reproduction test, along with determining the number of broods at each count. The *Ceriodaphnia dubia* test which would otherwise be terminated after 3 broods according to Section 13.12.1 of that method must be conducted for 8 days, with endpoints including survival, number of young per day, and number of broods at each recording interval. These readings are to be made at the end of the 6th, 7th and 8th day (specifically, at 144 hours, at 168 hours, and at 192 hours, respectively, from test initiation). This will be done assess the effect of that test acceptance criterion on test results. No test shall be terminated prior to the eighth day for any reason, including a failure to meet test acceptance criteria.

(11) If test results indicate too great of toxicity (i.e., effect is that all die in all concentrations) or not the expected toxicity (i.e., no effect at the highest concentration), the referee laboratories must investigate possible causes immediately, first by checking for transcription and calculation mistakes, and then by investigating possible contamination in dilution waters, organism cultures, equipment, or other procedural steps.

(12) Laboratories must perform all QA/QC tests listed Section 4 of the method manuals, and additional QA/QC for each organism/method manuals. Laboratories that purchase organisms must supply QA/QC from the test organism supplier and follow method manuals for the appropriate QA/QC for purchasing organisms.

(13) Data and statistical analyses must be submitted in the standardized hard copy and electronic format specified in Section 5 of this study plan. All bench sheets and raw data, including samples tracking and chemistry analysis data also must be submitted.

(14) Data analysis must be performed in accordance with the statistical programs specified in the methods manuals.

(15) An LC_{50} must be reported for each acute test and an NOEC for survival, and NOEC for growth/reproduction, an LC_{50}, and IC_{25} must be reported as appropriate for each short-term chronic test as described in the method manuals. The laboratories must report individual toxicity endpoints; laboratories are not allowed to average or perform other data manipulations unless required by a methods's instructions.

4.5.4 Method-Specific Requirements

The summary of test conditions for the twelve WET methods to be evaluated under Phase 5 for Rounds 1 - 3 are provided in Tables 8 - 19. These tables are extracted from the summary test condition tables in the methods manuals and modified to fit the scope of this study (see Section 4.5.3).

Round 1:

Table 8. Acute Test on the Fathead Minnow, *Pimephales promelas*. Summary of test conditions and test acceptability criteria for fathead minnow, *Pimephales promelas*, acute toxicity tests with effluents and receiving waters

1. Test type:	Static-renewal
2. Test duration:[1]	96 h
3. Temperature:	25 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 µE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	250 mL
8. Test solution volume:	200 mL
9. Renewal of test solutions:	At 48 h
10. Age of test organisms:	1-14 days; 24-h range in age
11. No. organisms per test chamber:	10
12. No. replicate chambers per concentration:	4
13. No. organisms per concentration:	40
14. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate 2 h prior to test solution renewal at 48 h
15. Test chamber cleaning:	Cleaning not required
16. Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water:	Moderately hard synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	Five concentrations and a control
19. Dilution factor	≥0.5
20. Endpoint:	Mortality (LC50)
21. Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period.
22. Sample volume required:	2 L for effluents
23. Test acceptability criterion:	90% or greater survival in controls

[1] Acute and chronic toxicity tests performed simultaneously to obtain acute/chronic ratios must use the same temperature and water hardness.

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 1:

Table 9. Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test. Summary of test conditions and test acceptability criteria for fathead minnow, *Pimephales promelas*, larval survival and growth toxicity tests with effluents and receiving waters

1. Test type:	Static renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Light intensity:	10-20 µE/m^2/s (50-100 ft-c) (ambient laboratory levels)
5. Photoperiod:	16 h light, 8 h darkness
6. Test chamber size:	500 mL
7. Test solution volume:	250 mL
8. Renewal of test solutions:	Daily
9. Age of test organisms:	Newly hatched larvae less than 24 h old. If shipped, not more than 48 h old, 24 h range in age
10. No. larvae per test chamber:	10
11. No. replicate chambers per concentration:	4
12. No. larvae per concentration:	40
13. Source of food:	Newly hatched *Artemia* nauplii (less than 24 h old)
14. Feeding regime:	Feed 0.1 g newly hatched (less than 24-h old) brine shrimp nauplii three times daily at 4-h intervals or, as a minimum, 0.15 g twice daily, 6 h between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient nauplii are added to provide an excess. Larvae fish are not fed during the final 12 h of the test
15. Cleaning:	Siphon daily, immediately before test solution renewal
16. Aeration:	None, unless DO concentration falls below 4.0 mg/L. Rate should not exceed 100 bubbles/min
17. Dilution water:	Moderately hard synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	Five concentrations and a control
19. Dilution factor	≥0.5
20. Test duration:	7 days
21. Endpoints:	Survival and growth (weight as mean per original)
22. Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chambers equals or exceeds 0.25 mg/surviving
23. Sampling requirements:	A minimum of three samples are collected on days one, three, and five with a maximum holding time of 36 h before first use (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
24. Sample volume required:	2.5 L/day of effluent

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 1:

Table 10. Cladoceran, *Ceriodaphnia dubia,* Acute Test. Summary of test conditions and test acceptability criteria for daphnid, *Ceriodaphnia dubia* acute toxicity tests with effluents and receiving waters

1. Test type:	Static renewal
2. Test duration:	48 h
3. Temperature:[1]	25 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 µE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	30 mL
8. Test solution volume:	15 mL
9. Renewal of test solutions:	None
10. Age of test organisms:	Less than 24-h old
11. No. organisms per test chamber:	5
12. No. replicate chambers per concentration:	4
13. No. organisms per concentration:	20
14. Feeding regime:	Feed YCT and *Selenastrum* while holding prior to the test; newly-released young should have food available a minimum of 2 h prior to use in a test.
15. Test chamber cleaning:	Cleaning not required
16. Test chamber aeration:	None
17. Dilution water:	Moderately hard synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	Five concentrations and a control
19. Dilution factor	≥0.5
20. Endpoint:	Mortality (LC50)
21. Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period.
22. Sample volume required:	1 L
23. Test acceptability criterion:	90% or greater survival in controls

[1] Acute and chronic toxicity tests performed simultaneously to obtain acute/chronic ratios must use the same temperature and water hardness.

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 1:

Table 11. Cladoceran, *Ceriodaphnia dubia,* Survival and Reproduction Test. Summary of test conditions and test acceptability criteria for daphnid, *Ceriodaphnia dubia*, survival and reproduction toxicity tests with effluents and receiving waters

1. Test type:	Static renewal
2. Temperature (°C):	25 ± 1°C
3. Light quality:	Ambient laboratory illumination
4. Light intensity:	10-20 µE/m^2/s, or 50-100 ft-c (ambient laboratory levels)
5. Photoperiod:	16 h light, 8 h dark
6. Test chamber size:	30 mL
7. Test solution volume:	15 mL
8. Renewal of test solutions:	Daily
9. Age of test organisms:	Less than 24 h; and all released within a 8-h period
10. No. neonates per test chamber:	1
11. No. replicate test chambers per concentration:	10
12. No. neonates per test concentration:	10
13. Feeding regime:	Feed 0.1 mL each of YCT and algal suspension per test chamber daily
14. Cleaning:	Use freshly cleaned glass beakers or new plastic cups daily
15. Aeration:	None
16. Dilution water:	Moderately hard synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW (see Methods Manual Section 7, Dilution Water)
17. Test concentrations:	Five concentrations and a control
18. Dilution factor:	≥0.5
19. Test duration:	Until 60% of surviving control organisms have three broods (maximum test duration 8 days)
20. Endpoints:	Survival and reproduction
21. Test acceptability criteria:	80% or greater survival and an average of 15 or more young per surviving female in the control solutions. 60% of surviving control organisms must produce three broods. Additional requirements in NOTE below.
22. Sampling requirements:	A minimum of three samples are collected on days one, three, and five with a maximum holding time of 36 h before first use (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
22. Sample volume required:	1 L/day

NOTE: Test vessels shall be randomized in accordance with the method manuals. In addition, block randomization and use of known parentage will be required for the *Ceriodaphnia* survival and reproduction test as described in the manual and guidance will be reiterated in the specific instructions provided to the laboratories. Daily observation of mortality and removal of dead organisms for each test is required, except for the *Selenastrum* test. Daily young counts are required for the *Ceriodaphnia dubia* survival and reproduction test, along with determining the number of broods at each count. The *Ceriodaphnia dubia* test which would otherwise be terminated after 3 broods according to methods manual Section 13.12.1 of that Method must be conducted for 8 days, with endpoints (survival and number of young per day and number of broods at each recording interval). These readings are to be made at the end of the 6th, 7th and 8th day (specifically, at 144 hours, at 168 hours, and at 192 hours, respectively, from test initiation). This will be done assess the effect of that test acceptance criterion on test results. No test shall be terminated prior to the eighth day for any reason, including a failure to meet test acceptance criteria.

Round 1:

Table 12. Green Alga, *Selenastrum capricornutum*, Growth Test. Summary of test conditions and test acceptability criteria for green alga, *Selenastrum capricornutum*, growth toxicity tests with effluents and receiving waters. Test will be conducted with EDTA and without EDTA.

1. Test type:	Static non-renewal
2. Temperature:	25 ± 1°C
3. Light quality:	"Cool white" fluorescent lighting
4. Light intensity:	86 ± 8.6 µE/m^2/s (400 ± 40 ft-c or 4306 lux)
5. Photoperiod:	Continuous illumination
6. Test chamber size:	125 mL or 250 mL
7. Test solution volume:	50 mL or 100 mL[1]
8. Renewal of test solutions:	None
9. Age of test organisms:	4 to 7 days
9. Initial cell density in test chambers:	10,000 cells/mL
10. No. replicate chambers per concentration:	4
11. Shaking rate:	100 cpm continuous, or twice daily by hand
12. Aeration:	None
13. Dilution water:	Algal stock culture medium, enriched uncontaminated source of synthetic water prepared using MILLIPORE MILLI-Q® or equivalent deionized water and reagent grade chemicals, or DMW (see Methods Manual Section 7, Dilution Water). One stock with EDTA and one without EDTA will be required for this study.
14. Test concentrations:	Five concentrations and a control
15. Test dilution factor:	≥0.5
16. Test duration:	96 h
17. Endpoint:	Growth (cell counts)
18. Test acceptability criteria:	1 X 10^6 cells/mL with EDTA or 2 X 10^5 cells/mL without EDTA in the controls; Variability of controls should not exceed 20%
19. Sampling requirements:	Grab or composite sample used within 36 h of completion of the sampling period (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
20. Sample volume required:	1 or 2 L depending on test volume

[1] For tests not continuously shaken use 25 mL in 125 mL flasks and 50 mL 250 mL flasks.

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 2:

Table 13. Sheepshead Minnow, *Cyprinodon variegatus*, Acute Test. Summary of test conditions and test acceptability criteria for sheepshead minnow, *Cyprinodon variegatus*, acute toxicity tests with effluents and receiving waters

1. Test type:	Static-renewal
2. Test duration:	96 h
3. Temperature:[1]	25°C ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 µE/m^2/s or (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	250 mL
8. Test solution volume:	200 mL
9. Renewal of test solutions:	At 48 h
10. Age of test organisms:	1-14 days; 24-h range in age
11. No. organisms per test chamber:	10
12. No. replicate chambers per concentration:	2
13. No. organisms per concentration:	20
14. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate 2 h prior to test solution renewal at 48 h
15. Test chamber cleaning:	Cleaning not required
16. Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water:	20 to 30 ‰ ± 2% Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	Five effluent concentrations and a control
19. Dilution factor	≥0.5
20. Endpoint:	Mortality (LC50)
21. Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period.
22. Sample volume required:	1 L for effluents
23. Test acceptability criterion:	90% or greater survival in controls
24. Salinity:	20 to 30 ‰ (±2% of the selected test salinity)

[1] Acute and chronic toxicity tests performed simultaneously to obtain acute/chronic ratios must use the same temperature and salinity.

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 2:

Table 14. Sheepshead Minnow, *Cyprinodon variegatus,* Larval Survival And Growth Test. Summary of test conditions and test acceptability criteria for the sheepshead minnow, *Cyprinodon variegatus*, larval survival and growth test with effluents and receiving waters

1. Test type:	Static renewal
2. Salinity:	20‰ to 32‰ (±2‰ of the selected test salinity)
3. Temperature:	25 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 µE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	600 mL beaker
8. Test solution volume:	500 mL/replicate (loading and DO restrictions must be met)
9. Renewal of test solutions:	Daily
10. Age of test organisms:	Newly hatched larvae (less than 24 h old; 24-h range in age)
11. No. larvae per test chamber:	10
12. No. replicate chambers per concentration:	4
13. No. larvae per concentration:	40
14. Source of food:	Newly hatched *Artemia* nauplii, (less than 24-h old)
15. Feeding regime:	Feed once a day 0.10 g wet weight *Artemia* nauplii per replicate on Days 0-2; Feed 0.15 g wet weight *Artemia* nauplii per replicate on Days 3-6
16. Cleaning:	Siphon daily, immediately before test solution renewal and feeding
17. Aeration:	None, unless DO falls below 4.0 mg/L, then aerate all chambers. Rate should be less than 100 bubbles/min
18. Dilution water:	Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water);
19. Test concentrations:	five concentrations and a control
20. Dilution factor:	≥0.5
21. Test duration:	7 days
22. Endpoints:	Survival and growth (weight)
23. Test acceptability criteria:	80% or greater survival in controls; average dry weight per surviving organism in control chambers should be 0.60 mg or greater, if unpreserved, or 0.50 mg or greater after no more than 7 days in 4% formalin or 70% ethanol
24. Sampling requirements:	A minimum of three samples are collected on days one, three, and five with a maximum holding time of 36 h before first use (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
25. Sample volume required:	6 L per day

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 2:

Table 15. Inland Silverside, *Menidia beryllina* , Acute Test. Summary of test conditions and test acceptability criteria for inland silverside, *Menidia beryllina*, acute toxicity test with effluents and receiving waters

1. Test type:	Static-renewal
2. Test duration:	96 h
3. Temperature:[1]	25°C ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 μE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	250 mL
8. Test solution volume:	200 mL
9. Renewal of test solutions:	At 48 h
10. Age of test organisms:	9-14 days; 24-h range in age
11. No. organisms per test chamber:	10
12. No. replicate chambers per concentration:	2
13. No. organisms per concentration:	20
14. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate 2 h prior to test solution renewal at 48 h
15. Test chamber cleaning:	Cleaning not required
16. Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water:	Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	five concentrations and a control
19. Dilution factor:	≥0.5
20. Endpoint:	Mortality (LC50)
21. Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period.
22. Sample volume required:	1 L for effluents
23. Test acceptability criterion:	90% or greater survival in controls
24. Salinity:	For *M. beryllina*: 5-32‰ (± 2% of the selected test salinity)

[1] Acute and chronic toxicity tests performed simultaneously to obtain acute/chronic ratios must use the same temperature and salinity

Round 2:

Table 16. Inland Silverside, *Menidia beryllina*, Larval Survival and Growth Test. Summary of test conditions and test acceptability criteria for the inland silverside, *Menidia beryllina*, larval survival and growth test with effluents and receiving waters

1. Test type:	Static renewal
2. Salinity:	5 to 32‰ (±2‰ of the selected test salinity)
3. Temperature:	25 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	l0-20 µE/m^2/s (50-100 ft-c) (Ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	1 L containers
8. Test solution volume:	750 mL/replicate (loading and DO restrictions must be met)
9. Renewal of test solutions:	Daily
10. Age of test organisms:	7-11 days post hatch; 24-h range in age
11. No. larvae per test chamber:	10
12. No. replicate chambers per concentration:	4
13. No. larvae per concentration:	40
14. Source of food:	Newly hatched *Artemia* nauplii (survival of 7-9 days old *Menidia beryllina* larvae improved by feeding 24 h old *Artemia*)
15. Feeding regime:	Feed 0.10 g wet weight *Artemia* nauplii per replicate on days 0-2; Feed 0.15 g wet weight *Artemia* nauplii per replicate on days 3-6
16. Cleaning:	Siphon daily, immediately before test solution renewal and feeding
17. Aeration:	None, unless DO concentration falls below 4.0 mg/L, then aerate all chambers. Rate should be less than 100 bubbles/min.
18. Dilution water:	Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
19. Test concentrations:	five concentrations and a control
20. Dilution factor:	≥0.5
21. Test duration:	7 days
22. Endpoints:	Survival and growth (weight as mean per original)
23. Test acceptability criteria:	80% or greater survival in controls, 0.50 mg average dry weight of control larvae where test starts with 7-days old larvae and dried immediately after test termination, or 0.43 mg or greater average dry weight per surviving control larvae, preserved not more than 7 days in 4% formalin or 70% ethanol
24. Sampling requirement:	A minimum of three samples are collected on days one, three, and five with a maximum holding time of 36 h before first use (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
25. Sample volume required:	6 L per day

Round 2:

Table 17. Mysid, *Holmesimysis costata*, Acute Test . The acute test procedure described in the Acute Methods Manual for *Mysidopsis bahia* will be used for this test with a salinity of 32% - 34% (± 2% of selected test salinity) and a temperature of 25 °C ± 1 °C.
Summary of test conditions and test acceptability criteria for mysid, *Holmesimysis costata*, acute toxicity tests with effluents and receiving waters

1. Test type:	Static-renewal
2. Test duration:	96 h
3. Temperature:	25°C ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 μE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness
7. Test chamber size:	250 mL
8. Test solution volume:	200 mL
9. Renewal of test solutions:	At 48 h
10. Age of test organisms:	1-5 days; 24-h range in age
11. No. organisms per test chamber:	10
12. No. replicate chambers per concentration:	4
13. No. organisms per concentration:	40
14. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; feed 0.2 mL of concentrated suspension of *Artemia* nauplii ≤ 24-h old, daily (approximately 100 nauplii per mysid)
15. Test chamber cleaning:	Cleaning not required
16. Test solution aeration:	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water:	Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
18. Test concentrations:	five concentrations and a control
19. Dilution factor:	≥0.5
20. Endpoint:	Mortality (LC50)
21. Sampling and sample holding requirements:	Grab or composite samples are used within 36 h of completion of the sampling period.
22. Sample volume required:	1 L for effluents
23. Test acceptability criterion:	90% or greater survival in controls
24. Salinity:	32-34‰ (±2%of the selected test salinity)

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 3:

Table 18. Mysid Shrimp, *Mysidopsis bahia,* Acute and Survival , Growth , and Fecundity Test. Summary of test conditions and test acceptability criteria for the mysid, *Mysidopsis bahia*, seven day survival, growth, and fecundity test with effluents and receiving waters

1. Test type:	Static renewal, nested acute
2. Salinity:	20‰ to 30‰ (±2‰ of the selected test salinity)
3. Temperature:	26 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 µE/m^2/s (50-100 ft-c.) (ambient laboratory levels)
6. Photoperiod:	16 h light, 8 h darkness, with phase in/out period
7. Test chamber:	8 oz plastic disposable cups, or 400 mL glass beakers
8. Test solution volume:	150 mL per replicate
9. Renewal of test solutions:	Daily
10. Age of test organisms:	7 days (survival readings daily)
11. No. organisms per test chamber:	5
12. No. replicate chambers per concentration:	8
13. No. larvae per concentration:	40
14. Source of food:	Newly hatched *Artemia* nauplii (less than 24 h old)
15. Feeding regime:	Feed 150 24 h old nauplii per mysid daily, half after test solution renewal and half after 8-12 h.
16. Cleaning:	Pipette excess food from cups daily immediately before test solution renewal and feeding.
17. Aeration:	None unless DO falls below 4.0 mg/L, then gently aerate in all cups
18. Dilution water:	Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
19. Test concentrations:	five concentrations and a control
20. Dilution factor:	≥0.5
21. Test duration:	7 days
22. Endpoints:	Survival, growth, and egg development
23. Test acceptability criteria:	Acute: 90% survival Chronic: 80% or greater survival, average dry weight 0.20 mg or greater in controls; fecundity may be used if 50% or more of females in controls produce eggs
24. Sampling requirements:	A minimum of three samples are collected on days one, three, and five with a maximum holding time of 36 h before first use (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
25. Sample volume required:	3 L per day

NOTE: Test vessels shall be randomized in accordance with the method manuals.

Round 3:

Table 19. Red Macroalga, *Champia parvula*, Reproduction Test. Summary of test conditions and test acceptability criteria for the red macroalga, *Champia parvula*, sexual reproduction test

1. Test type:	Static non-renewal
2. Salinity:	30‰ (± 2 of the selected test salinity)
3. Temperature:	23 ± 1°C
4. Photoperiod:	16 h light, 8 h darkness
5. Light intensity:	75 µE/m^2/s (500 ft-c)
6. Light source:	Cool-white fluorescent lights
7. Test chamber size:	200 mL polystyrene cups, or 250 mL Erlenmeyer flasks
8. Test solution volume:	100 mL
9. No. organisms per test chamber:	5 female branch tips and 1 male plant
10. No. replicate chambers per concentration:	4
11. No. organisms per concentrations:	24
12. Dilution water:	30‰ ± 2% Modified Forty Fathoms®, HW Marinemix®, GP2 or equivalent, artificial seawater prepared with MILLIPORE MILLI-Q® or equivalent deionized water (see Methods Manual Section 7, Dilution Water)
13. Test concentrations:	five concentrations and a control
14. Test dilution factor:	≥0.5
15. Test duration:	2 day exposure to effluent, followed by 5 to 7-day recovery period in control medium for cyctocarp development
16. Endpoints:	Reduction in cystocarp production compared to controls
17. Test acceptability criteria:	80% or greater survival, and an average of 10 cystocarps per plant in controls
18. Sampling requirements:	Grab or composite sample used within 36 h of completion of the sampling period (see Methods Manual Section 8, Effluent and Receiving Water Sampling, Sample Handling and Sample Preparation for Toxicity Tests, Subsection 8.5.4)
19. Sample volume required:	2 L per test

NOTE: Test vessels shall be randomized in accordance with the method manuals.

SECTION 5: DATA REPORTING AND EVALUATION

Each referee and participant laboratory will be required to submit data reports in a hard copy format that is consistent with the applicable methods manual. At a minimum, this report should follow the data reporting format outlined in Table 20 and include all laboratory bench sheets. Laboratories also will be required to submit selected data in an electronic format (Excel spreadsheet, or equivalent) that will allow SCC to create a database of study results. This database will facilitate automated review and statistical analysis of study results. Specific instructions regarding the electronic format will be provided to referee and participant laboratories prior to study initiation. All raw data will be made available in the public record.

Upon receipt of each laboratory data package, SCC will review the results to ensure that they were generated in accordance with the required procedures. Data generated by all qualified participating laboratories shall be considered in the evaluation of the test methods and will be compiled in a study database and statistically analyzed (e.g., analysis of outliers) to determine the intralaboratory and interlaboratory variability of the acute and short-term chronic methods under study. Data will also be assessed to determine the success rate for test initiation and test completion for each method and the rate at which the tests indicate "toxicity" is present when measuring non-toxic samples. Overall, EPA will evaluate the study results to draw conclusions about the performance of standardized WET tests. Participant laboratories that fail to initiate tests in Phase 5 will not be included in the success rate calculations nor statistical analyses. SCC will assemble background information and study data into a final study report for review by EPA staff.

EPA shall evaluate results from the interlaboratory study in accordance with the criteria for evaluating the adequacy of biological methods described in "Availability, Adequacy, and Comparability for the Analysis of Pollutants Established Under Section 304(h) of the Federal Water Pollution Control Act," EPA/600/9-87/030 (September 1988), and, to the extent applicable, the "Data Quality Objectives" guidance (from EPA's Permit Writers' Guide dated November 1990 and Guidance for Planning for Data Collection, EPA/QA/G-4).

Note: Laboratories may not independently publish the results of analyses they are paid to perform under this study plan.

Table 20 - Data Reporting Format

Section 1 - Summary Page

1.1 Laboratory name
1.2 Laboratory address and phone number
1.3 Name and signature of laboratory QA Officer, certifying that data have been internally reviewed and that personnel meticously followed the methods and the procedures are deemed to be compliant with the methods and acceptable for reporting purposes.
1.4 Laboratory contact responsible for study
1.5 Analyst who performed WET tests (full names)
1.6 Toxicity tests performed
1.7 Detailed explanations of any difficulties encountered and any approved modifications to the techniques specified in the study plan, SOWs, specific instructions, or the methods manuals.
1.8 Number of successful tests completed

Section 2 - Sample Information

2.1 Number of samples received and EPA sample number assigned to each sample
2.2. Dates of sample receipt
2.3 Sample temperature when received at laboratory
2.4 Physical and chemical data of sample contents (as required in appropriate method)
2.5 Dilution water

2.5.1 Source and time frame water is used or how maintained
2.5.2 Collection or Preparation date(s), where applicable
2.5.3 Pretreatment information
2.5.4 Physical and chemical characteristics (pH, hardness, conductivity, salinity, etc.)

2.6 Sample storage information
2.7 Sample preparation for testing information

Section 3 - Test Conditions

3.1 Toxicity test method used (title, number, source)
3.2 Endpoint(s) of test(s)
3.3 Deviations from reference method(s), if any, and reason(s)
3.4 Date and time test(s) started, date and time samples were prepared and solutions transferred for renewals.
3.5 Date and time test(s) terminated
3.6 Type and volume of test chambers
3.7 Volume of solution used per chamber
3.8 Number of organisms per test chamber
3.9 Number of replicate test chambers per treatment
3.10 Feeding frequency and amount and type of food (be specific with sources, concentrations of foods (i.e, algae concentration, YCT solids level, preparation dates)
3.11 Acclimation of test organisms (temperature mean and range and, where applicable, salinity mean and range)
3.12 Test temperature (mean and range)
3.13 Test salinity, where applicable (mean and range)
3.14 Specify if aeration was needed
3.15 Specify if organisms were dried immediately for weighing or preserved prior to drying
3.16 Specify how food was prepared and sources of food
3.17 Describe how routine chemistries on new solutions were made (in actual test chamber or in beakers after dispensing)
3.18 Describe how randomization was conducted, especially blocking and known parentage. Report how brood distinctions were made and male (if any) identification was made.

Section 4 - Test Organisms

4.1 Scientific name of test species, verification of species documented
4.2 Age (life stage) of test species, be specific for all species; Age at time of test initiation. Be specific, for example for *C. dubia* be sure to clarify the window of age of the neonates as well as the overall age of the animals.
4.3 Mean length and weight (where applicable)
4.4 Source and QA/QC test conditions
4.5 Holding Conditions
4.6 Diseases and treatment (where applicable)
4.7 Taxonomic key used for species identification

Section 5 - Quality Assurance

5.1 Reference toxicant used routinely; source; date received; lot number
5.2 Date and time of most recent reference toxicant test; test results and current control (cusum) chart including 20 most recent data points
5.3 Dilution water used in reference toxicant tests (with characteristics provided)
5.4 Physical and chemical methods used
5.5 Results (NOEC, IC_{25}, or LC_{50} where applicable, LOEC or EC_{50})

Section 6 - Results

6.1 Copies of all bench sheets. Be sure to count and notate broods for reproduction test with *Ceriodaphnia*
6.2 Raw toxicity data in tabular form, including daily records of affected organisms in each replicate at each concentration (including controls) and plots of toxicity data or concentration vs. biological response
6.3 Table of endpoints (LC_{50}, IC_{25}, NOEC for each endpoint) and confidence limits (where applicable)
6.4 Statistical methods used to calculate endpoints
6.5 Summary table of physical and chemical data

SECTION 6: LABORATORY PREQUALIFICATION REQUIREMENTS

6.1 Summary of Prequalification Requirements

Prequalification of referee and participant laboratories will be conducted in two parts as summarized in Section 4.2 of this document. In Part I of prequalification, each laboratory must submit recent data and further background information to EPA for consideration as given below. From these data submissions, EPA will develop lists of laboratories that will be asked to take part in Part II of prequalification through the performance of a prequalification test for each species/test method for which data was submitted in Part I and the submission of general and WET method specific information regarding the capabilities of potential laboratories. A "laboratory prequalification document" that provides specific instructions for prequalification will be distributed to potential laboratories prior to initiation of Part I prequalification.

A detailed description of Part I and II prequalification requirements, rejection criteria, and turnaround requirements are provided in Sections 6.2 - 6.4 below.

6.2 Part I - Historical WET Testing Experience and Proficiency

Each potential referee or participant laboratory **must** demonstrate the following for Part I of prequalification:

(1) Combination of facilities, equipment, staff and laboratory capabilities are sufficient to meet study needs. Detailed information must be provided on the type of laboratory, sample handling, resumes of laboratory staff participating in the study, temperature control, source of water, type of food and source of foods, test equipment used for conduct of each test method to evaluate, source of organisms, and quality control measures for each organism.

(2) Each laboratory must provide an internal Standard Operating Procedure (SOP) that has been in use at the laboratory for each method to be performed. Internal laboratory SOPs for each test method must be in place with dates of origination of SOP. Supporting internal laboratory SOPs for culture, food preparation, dilution water preparation for each species and each method must be included for the laboratory to prequalify.

(3) Evidence that reference toxicant tests are conducted at the appropriate frequency (e.g., monthly for tests that are routinely run for NPDES permits) and a control chart that includes these reference toxicant tests. For instance, the method 1002 (*Ceriodaphnia dubia 3-brood* test must have the reference toxicant data for the same method at the same temperature If more than one reference toxicant is used for each species and method, a laboratory need only provide data for one reference toxicant per method. If data charts have been in place but new source of organisms requires new charts, provide the charts along with an explanation.

(4) Evidence that the laboratory maintains control (cusum) charts for reference toxicant tests, and raw data (actual data sheets, summarized and data analysis summary provided) for each data point must be provided. These data control charts must cover 12-24 data points each. Data charts with NOEC and/or IC_{25} for the same test values should be provided or describe why one is used rather than the other. Explanations must be included if methods used to develop control charts using reference toxicants deviate from promulgated methods or from the previous edition of a relevant test protocol.

(5) Laboratory can demonstrate evidence of corrective action for any toxicity test endpoint value that falls outside the control limits. Each laboratory must include their internal SOP for determining the

control chart method; which includes the equation and method for maintaining chart with 20 most current data points.

(6) Each laboratory must include a narrative explanation on the width of the control limits for the laboratory.

(7) Each laboratory must provide the results of the most recent Discharge Monitoring Requirement Quality Assurance (DMRQA) study, if the lab participated. The laboratory must also readily provide data point(s) for each method performed for the DMRQA study from the last year (1997). If laboratory did not participate a narrative statement to that effect must be included.

Rejection of laboratories would be based on the following:

(1) Data did not meet test acceptability criteria in the provided specific instructions and methods manuals; and laboratory certified the tests were conducted following the manual specifications.

(2) Combination of facilities, equipment, staff and lab capabilities were insufficient to meet study needs.

(3) Internal laboratory SOP's for each method are vague and cannot be discerned and/or are generally insufficient to support performance of the methods in accordance with specific instructions provided by EPA. The laboratory did not follow those internal laboratory SOPs submitted in Part I above, or if an update of the SOP from Part I above is provided, then a description of why and how the test protocol changed was not included.

(4) Reference toxicant test(s) were not conducted at the appropriate frequency (monthly for tests that are routinely run for permits) and a satisfactory explanation was provided.

(5) Control charts not maintained for reference toxicant tests, data not provided (cusum chart for each endpoint and raw data for each data point). These control charts must cover 12-24 data points for each species and test method.

(6) No explanation or evidence of corrective action is provided for any value falls outside the control limits

(7) Laboratory did not provide the most recent DMRQA study results and an explanation for non-passing results was not provided. If the laboratory did not participate in the DMRQA study, the laboratory did not include an explanation as to why they didn’t participate.

6.3 Part II - Evaluation and Acceptance of Prequalification Test Data

Once selected participant laboratories have conducted the prequalification test for each method they intend to perform, the data must be summarized according to the instructions provided with the sample. Successful completion of the prequalification sample will include:

(1) Adherence to the specific criteria for each WET method given in Tables 8 - 19 in Section 4.5.4 of this study plan, internal laboratory SOPs, specific instructions, and any additional guidance provided to laboratories with prequalification samples. Confirmation that the results met test acceptability criteria. For chronic tests, the test acceptability criteria must be met for all required endpoints (e.g., survival, growth, reproduction, or fecundity). Specific study parameters must follow those provided in Tables 8 - 19.

(2) Data is reported in the specified format and all relevant data is provided.

(3) Data evaluation: Reported statistical endpoints, LC_{50}, IC_{25}, NOEC for survival, NOEC for growth/reproduction are reported and conducted according to promulgated methods. Supporting documentation of analysis is provided along with all raw data. All prequalification results with each WET method will be evaluated for outliers as a group. Laboratories will be given the opportunity to explain outlier values.

NOTE: In lieu of a prequalification test, referee laboratories must submit at least three client recommendations for each WET test the laboratory is seeking to support in the study. These recommendations must come from clients for whom they have conducted these same WET tests in the past. The recommendations should demonstrate that the laboratory is qualified to support the WET tests for which information was submitted during Part I prequalification.

Rejection of prequalification data submitted by potential participant laboratories would be based on the following:

(1) Data did not meet test acceptability criteria in the internal laboratory SOP, specific instructions provided for the study, and methods manuals.

(2) Laboratory did not follow those internal laboratory SOPs submitted in Part I above, or if an update of the SOP submitted in Part I above is provided, then a description of why and how the test protocol changed was not included.

(3) Reference toxicant test(s), QA/QC tests are not conducted at the appropriate frequency (monthly for tests that are routinely run for permits), not performed using the test method(s) they are prequalifying to perform in the study, or satisfactory explanation is not provided for employing a different test frequency or test method. For instance, the method 1002 (*Ceriodaphnia dubia* 3-brood test must have the reference toxicant data for the same method at the same temperature.

(4) Statistical analysis did not follow recommended guidance; supporting documentation of analysis is not provided.

(5) Satisfactory explanation of outlier prequalification results were not provided.

Rejection of WET testing recommendations submitted by potential referee laboratories would be based on the following:

(1) Three client recommendations were not submitted for each WET method the prequalifying laboratory is seeking to support.

(2) For an individual method, submitted recommendations were prepared by entities for whom the prequalifying laboratory has not conducted the individual WET method.

6.4 Prequalification Information Turnaround Requirements

Specific data turnaround and submission requirements will be provided to prequalifying laboratories prior to initiation of Part I prequalification. All required prequalification information must be received by EPA in accordance with the final turnaround requirements to be considered valid.

- Potential participant and referee laboratories must submit Part I prequalification information and the bid response sheet to EPA within 15 business days of receipt of the solicitation package.

- Potential participant laboratories must submit results of Part II prequalification test(s) in hard copy and electronic format to EPA within 21 business days of receipt of prequalification sample(s).
- Potential referee laboratories must submit WET testing recommendations within 21 business days of notification that they have been asked to participate in Part II prequalification.